Original Article

Role of blood grouping as a prognostic marker in breast carcinoma its relationship with histological and hormonal prognostic markers

Lokesh Haswani, T. N. Suresh, A. Hemalatha, M. L. Harendra Kumar, A. Bhaskaran[1]

Departments Pathology and [1]Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

ABSTRACT

Context: Breast carcinomas are one of the leading causes of mortality and morbidity in our country. Estrogen receptor (ER) and progesterone receptor (PR) status plays a very important role in therapeutic decisions in managing these patients. ABO and Rh blood type has been associated with risk and survival for several malignancies. **Aims and Objectives:** To know the frequency of ER and PR positivity status in the semi-urban population. To relate ABO/Rh blood group, ER and PR status with histopathological stage and Nottingham prognostic index (NPI). **Materials and Methods:** This was a retrospective study carried out on 45 cases from July 2012 to December 2013 who underwent mastectomy for breast cancer were included in our study. Histopathological grade of the tumor, lymph node invasion was noted. NPI was calculated. Immunohistochemistry was done using antibodies against ER and PR. Blood grouping and Rh typing was done. Descriptive statistics and Chi-square tests were done using SPSS package 20. $P < 0.05$ was considered to be significant. **Results:** In our study, maximum number of cases were in the fourth decade of life with a mean age of 52 years. ER and PRs were positive in 23/45 (51.1%) of cases. Most of the ER and PR negative patients were in the premenopausal group. Lymph node-positive tumors were ER negative (54%) and PR negative (58%). Patients in our study belonged to Group B (35.5%) and Group O (35.5%). Eighty percent of Rh negative cases were ER and PR positive. A 2 × 2 table correlating ER and PR positivity with Rh negative status revealed a positive correlation with $P < 0.05$. Majority of ER and PR negative tumors belonged to Groups B and O. **Conclusion:** Majority of the patients were in premenopausal age group with 51.1% of our cases were ER and PR positive. Majority of Rh negative17 patients were ER and PR positive.

Key words: Blood group, breast carcinoma, estrogen receptor, progesterone receptor status

INTRODUCTION

Breast carcinomas are the second most common cause of cancer death in female's occurring worldwide. Every year 100,000 new breast cancer patients are being detected in India.[1] Numerous conventional markers such as tumor size, histological type, differentiation, microscopic grade, lymph node status, tumor necrosis, and hormone receptor status are used in assessing the prognostic and therapeutic outcome in these patients with carcinoma breast.[2,3]

Access this article online	
Quick Response Code:	Website: www.ccij-online.org
	DOI: 10.4103/2278-0513.142628

Though numerous grading systems have been used in grading carcinoma breast, modified Bloom–Richardson grading system is routinely followed as it correlates with aggressiveness of tumor.[4]

The Nottingham prognostic index (NPI), which incorporates tumor size, histological grade and lymph node status, is established as the most useful means of stratifying patients with invasive breast carcinoma for prognostic, therapeutic management and survival.[5]

The therapeutic benefits of estrogen receptor (ER) and progesterone receptor (PR) assay is well documented worldwide. Immunohistochemical demonstration of ER and PR receptors is convenient, cost-effective, and can be done on paraffin blocks. When done is also one of the important known prognostic factors used in the treatment.[4,6]

ABO and Rh blood type has been associated with risk and survival for several malignancies. ABO blood group genes

Address for correspondence: Dr. A. Hemalatha, Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India.
E-mail: drhemashashi@gmail.com

are mapped at 9q34.2 region. The Rh gene locus is known to be located on the short arm of chromosome 1 (lp36.2). Genetic alterations are known to occur in these regions of chromosome 1 and 9 in several cancers.[7]

This has made us to take up this study with aims and objectives:

- To know the frequency of ER and PR positivity status in the semi-urban population of Kolar
- To relate ABO/Rh blood group, ER and PR status with histopathological stage and NPI.

MATERIALS AND METHODS

This was a retrospective study done from July 2012 to December 2013 on patients who underwent mastectomy for breast cancer. Study was started after taking ethical clearance from our Institutional Ethics Committee.

Inclusive criteria

Forty-five cases of infiltrating ductal carcinoma were included in our study.

Exclusive criteria

Male breast carcinomas and sarcomas were excluded from our study.

Histopathology analysis

Demographic details such as age, site and side of the tumor were noted from the case file. Tumor size and lymph node involvement was noted from the gross specimen. After grossing the tumor according to standard protocol, sections of 4–6 µm were cut from paraffin blocks and stained with hematoxylin and eosin stain. These sections were analyzed by two different pathologists and parameters such as histopathological grade of the tumor, lymph node invasion were noted. Nottingham prognostic index was calculated using the formula - NPI = 0.2 × size + grade + nodal status. All the cases were divided into three groups based on NPI score NPI < 3.4 - low risk, between 3.4 and 5.4 intermediate risk, >5.4 is considered high risk.[4]

Immunohistochemistry analysis

Sections of 4 µm were cut from blocks of tumor proper, without any necrosis, hemorrhage for immunohistochemistry. IHC was done using antibodies against ER and PR by horse radish peroxidase anti peroxidase method using both primary and secondary antibodies from Biogenix USA. Slides were first deparaffinized using various degrees of alcohol and xylene, followed by antigen retrieval in citrate buffer of pH 6 using microwave. These slides were then incubated in peroxidase block, followed by incubation in the power block. Slides were then incubated in primary and secondary antibodies. pH of the entire procedure was maintained by washing the slides with Tris buffer sulfate wash buffer of 7.6 pH. Staining of the antigen-antibody complex was done using diaminobenzidine. Slides were then counterstained with hematoxylin. Positive and negative controls were run simultaneously. Distinct nuclear staining in both ER and PR stained sections of tumor proper were considered to be positive. [Figure 1, Figure 2].

Scoring of both ER, PR were done using Allred scoring.[8]

HER-2/neu was not done in any of our patients.

Blood grouping

Blood grouping was done by slide method using anti A, anti B and anti Rh antibodies. A case with doubtful agglutination was confirmed under microscope.

In doubtful cases, blood grouping was done by tube method.

Statistical analysis

Descriptive statistics and Chi-square tests were performed using software package used for statistical analysis (SPSS), USA package 20. $P < 0.05$ was considered to be significant.

RESULTS

A total of 45 cases of infiltrating ductal carcinoma cases was taken up for the study. Histopathological analysis, blood grouping and ER, PR status were analyzed in all these cases.

Age distribution

Maximum numbers of cases are in the age group of 41–50 years with a mean age of 52 years. Age distributions of cases are shown in the pie chart [Chart 1].

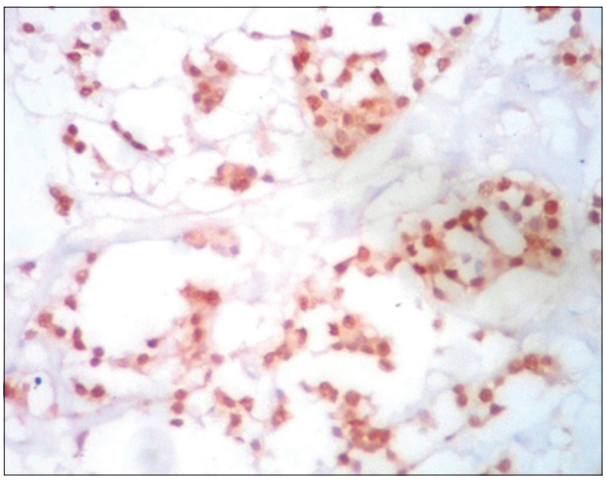

Figure 1: Immunohistochemistry for estrogen receptor (Allred score - 8)

Estrogen receptor, progesterone receptor status and age

Estrogen receptor and PRs were positive in 23/45 (51.1%) of cases. The distribution of ER and PR status with respect to the age of the patient is represented in Table 1.

Most of the ER and PR negative patients were in the premenopausal group.

Estrogen receptor, progesterone receptor status and Nottingham prognostic index

For statistical convenience NPI was divided into two groups' low NPI and high NPI. The details are as mentioned in Table 2.

Majority of the ER and PR positive cells belonged to low NPI group, which was statistically significant ($P < 0.05$). Six patients of ER positivity and seven patients of PR positivity had high NPI.

Estrogen receptor, progesterone receptor status and lymph node metastasis

The comparison between ER and PR status and lymph node involvement is shown in Table 3.

Majority of lymph node positive tumors were ER negative (54%) and PR negative (58%). However, we did not reach any statistical significance regarding the lymph node status and ER and PR positivity.

Estrogen receptor progesterone receptor status and blood grouping

In our study, majority of the patients belonged to Group B (35.5%) and Group O (35.5%). Majority of ER and PR negative tumors belonged to Groups B and O. The distribution of ER, PR status and blood group is in the Table 4. However, no statistical significance was seen between ER and PR status and blood group.

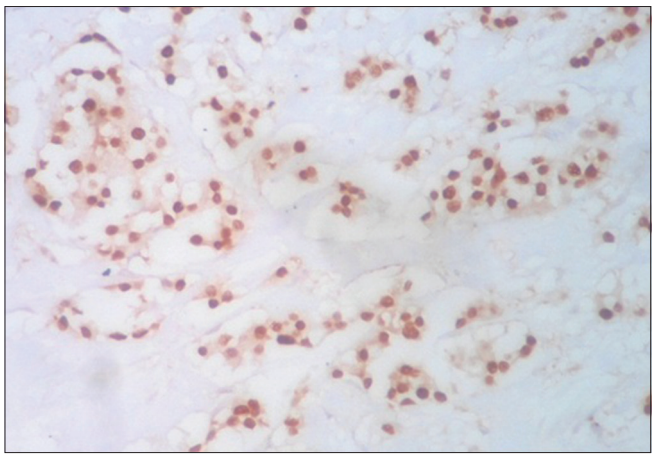

Figure 2: Immunohistochemistry for progesterone receptor (Allred score - 8)

8/10 Rh negative cases were ER and PR positive. A 2 × 2 table correlating ER and PR positivity with Rh negative status revealed a positive correlation with $P < 0.05$.

DISCUSSION

Breast carcinomas are leading the cause of mortality and morbidity in female patients in our country. An

Table 1: The distribution of ER and PR status according to age

Age (years)	ER positive	ER negative	PR positive	PR negative
<50	11	16	12	15
>50	12	6	11	7

ER: Estrogen receptor, PR: Progesterone receptor

Table 2: ER, PR positive with NPI

NPI	Total	ER positive	ER negative	PR positive	PR negative
Low	27	17	10	16	11
High	18	6	12	7	11

ER: Estrogen receptor, PR: Progesterone receptor, NPI: Nottingham prognostic index

Table 3: Comparison between ER, PR status and lymph node involvement

Lymphnode status	Total no of cases	ER positive	ER negative	PR positive	PR negative
Positive	26	12	14	11	15
Negative	19	11	8	12	7

ER: Estrogen receptor, PR: Progesterone receptor

Table 4: Correlation between blood group and ER, PR status

Blood group	ER positive	ER negative	PR positive	PR negative
A	6	2	5	3
B	3	2	3	2
AB	9	7	10	6
O	5	11	5	11

ER: Estrogen receptor, PR: Progesterone receptor

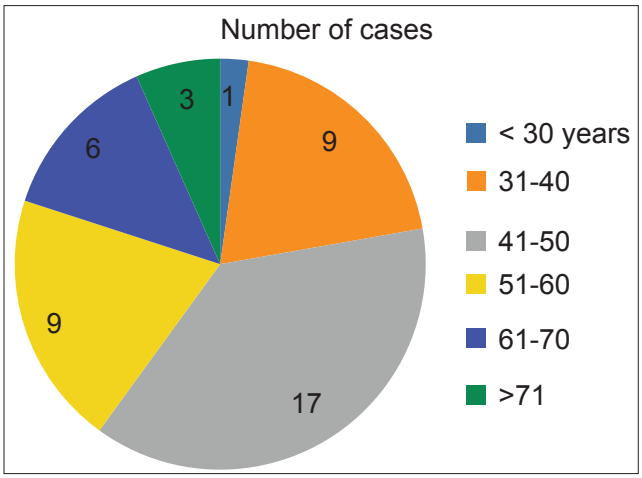

Chart 1: Age distribution of cases

individuals blood group is determined by presence of absence of glycosyltransferase. In breast carcinomas, at cellular level glycosylation patterns of these sugars, over expression of N linked beta, six branched oligosaccharides is known to cause alterations in cell adhesion and migration.[9-13]

Age plays an important risk factor in pathogenesis of breast carcinomas

In our study majority of carcinoma patients were in the age group of 41–50 years (17/45, 37.8%) with 60% of the patients in premenopausal age group. This kind of distribution was also found by Desai *et al*. wherein majority of the patients were in 63.4% of patients were <60 years of age.[14]

This finding reconfirms the fact that most of the patients in Indian subcontinent develop breast carcinomas at an earlier age, emphasizing the fact that early diagnosis is the key to prevent widespread malignancy and metastasis.

Hormone receptor ER and PR acts as a predictor of response to therapy and overall survival of patients. Hence, it becomes important to score these receptors in all cases of breast carcinomas. Both ER and PR was positive in 23/45 (51.1%) of patients. In a study in Nepal population ER was positive in 28% of their population, while PR was positive in 19% of cases.[15] In Chinese population ER was positive in 73.5% of the population, while PR was positive in 65.5% of their population. However our findings correlated with findings by Desai *et al*. who have described ER positivity in 46% of tumors, PR positivity in 46.1% of tumors. This variation clearly suggests the role of race in oncogeneseis and response to different therapeutic outcomes.[14,16] HER-2/neu immunohistochemistry was not done in our patients because of the cost involved in the diagnosis of ambiguous cases and therapy of positive cases.

Twelve patients with ER positivity were older than 50 years, while 11 patients were PR positive. No difference of age was found in ER and PR positivity. However, premenopausal had higher ER and PR negative cases, similar results were found by Sharif *et al*. In a study by Klimant *et al*. ER and PR was more positive in premenopausal women. Though we did not reach any statistical significance regarding ER, PR status and age, it has been postulated that premenopausal women in the western region have more ER, PR positive status and postmenopausal women in Asian population have higher ER, PR status.

In our population, breast carcinoma occurs a decade earlier than the west. These young patients are known to have higher levels of estrogen and progesterone hormones in serum and correspondingly low expression of steroid receptors in the tumors.[2,14,17]

In our study, 45% of cases with lymph node positive status were ER and PR negative. Similar results were found by Sharif *et al*.[2] reaffirming the hypothesis that tumor may lose their antigenicity when they acquire metastatic potential.

For statistical convenience NPI was divided into two categories low and high. More number of high NPI tumors were ER and PR negative which was statistically significant ($P < 0.05$). Similar findings have been described by Van Belle *et al*., where in addition of PR and HER-2/neu to NPI increases the calculation of 5 years prognostic accuracy.[4]

Holdsworth *et al*. study first described that blood group was a prognostic indicator in the breast cancer, and it may have some implications in survival of these patients. He concluded that it is important to have knowledge of relational patterns between blood group and hormone status of carcinomas as these blood group patterns may relate to the survival outcome in patients with breast carcinoma.[13]

In our study, majority of the patients with carcinoma belonged to Group B (35.5%) and Group O (35.5%). Ichikawa *et al*. has suggested that lack of A antigen expression in combination with expression of oncogenes such as p53 was present an increase in cell motility, resistance to apoptosis and increase in cells that are proliferative, undifferentiated, with increased capacity to evade immune surveillance due to loss of differentiation markers were noted.[18] Terminal glycosylation pattern of blood Group A antigen does not involve β1,6 oligosaccharides but rather consists of H antigen termination in a α1-3 Gal Nac residue accomplished by glycosyltransferases, different from those mediation β1,6 glycosylation. This specific class of glycosyltransferases are more susceptible during malignant transformation.[17]

However study in Greek women, infiltrating ductal carcinoma of the breast were more associated with A blood group and less with blood Group AB.[19] In a study by Ichikawa *et al*. Blood Group A was protective against breast carcinoma.[18]

Majority of the tumors in our study were of Grade II (28/45), majority of these tumors belonged to Group B (10/28) and Group O (10/28), among 12/45 tumors were of Grade III, 6/12 were Group B and 3/12 were Group O.

We did not reach any statistical significance between ER and PR status and blood group. Klimant *et al*. also did not find any correlation between ER and PR and HER-2/neu status with blood group. About 80% of Rh negative cases were ER and PR positive. A 2 × 2 table correlating ER and PR positivity with Rh negative status revealed a positive

correlation with $P < 0.05$. Rh positive have poor prognosis as compared to Rh negative patients in our study.

However, our sample size was small to arrive at specific hypothesis regarding Rh positivity/negativity, and ER PR status, hence similar study with a larger group of the population is needed.

CONCLUSION

Majority of the patients were in premenopausal age group. About 51.1% of our cases were ER and PR positive, ER and PR positivity were associated with lymph node negative status and correlated with low NPI status. Most of the carcinoma patients were blood Groups B and O and was associated with Grades II and III. Majority of Rh negative patients were ER and PR positive. No correlation was found between ER and PR status and blood group of the patient.

REFERENCES

1. Ali I, Waseem A, Saleem W, Saleem K. Cancer scenario in India with future perspectives. Cancer Ther 2011;8:56-70.
2. Sharif MA, Mamoon N, Mushtaq S, Khadim MT, Jamal S. Steroid hormone receptor association with prognostic markers in breast carcinoma in Northern Pakistan. J Coll Physicians Surg Pak 2010;20:181-5.
3. Thorat MA, Badve S. Prognostic factors in invasive breast carcinoma: Do new molecular techniques/profiling add significantly to traditional histological factor? Curr Diagn Pathol 2007;13:116-25.
4. Van Belle V, Van Calster B, Brouckaert O, Vanden Bempt I, Pintens S, Harvey V, *et al.* Qualitative assessment of the progesterone receptor and HER2 improves the Nottingham prognostic index up to 5 years after breast cancer diagnosis. J Clin Oncol 2010;28:4129-34.
5. Frkovic-Grazio S, Bracko M. Long term prognostic value of Nottingham histological grade and its components in early (pT1N0M0) breast carcinoma. J Clin Pathol 2002;55:88-92.
6. Zafrani B, Aubriot MH, Mouret E, De Crémoux P, De Rycke Y, Nicolas A, *et al.* High sensitivity and specificity of immunohistochemistry for the detection of hormone receptors in breast carcinoma: Comparison with biochemical determination in a prospective study of 793 cases. Histopathology 2000;37:536-45.
7. Gularia K, Singh HP, Kaur H, Sambyal V. ABO blood groups in gastrointestinal tract (GIT) and breast carcinoma patients. Anthropologist 2005;7:189-92.
8. Allred DC, Bustamante MA, Daniel CO, Gaskill HV, Cruz AB Jr. Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Evaluation of 130 cases and review of the literature regarding concordance with biochemical assay and clinical relevance. Arch Surg 1990;125:107-13.
9. Feizi T. The major blood Group ABO (H) determining genes are isolated. Trends Biochem Sci 1990;15:330-1.
10. Dennis JW, Laferté S. Oncodevelopmental expression of – GlcNAc beta 1-6Man alpha 1-6Man beta 1 – branched asparagine-linked oligosaccharides in murine tissues and human breast carcinomas. Cancer Res 1989;49:945-50.
11. Demetriou M, Nabi IR, Coppolino M, Dedhar S, Dennis JW. Reduced contact-inhibition and substratum adhesion in epithelial cells expressing GlcNAc-transferase V. J Cell Biol 1995;130:383-92.
12. Narita T, Funahashi H, Satoh Y, Watanabe T, Sakamoto J, Takagi H. Association of expression of blood group-related carbohydrate antigens with prognosis in breast cancer. Cancer 1993;71:3044-53.
13. Holdsworth PJ, Thorogood J, Benson EA, Clayden AD. Blood group as a prognostic indicator in breast cancer. Br Med J (Clin Res Ed) 1985;290:671-3.
14. Desai SB, Moonim MT, Gill AK, Punia RS, Naresh KN, Chinoy RF. Hormone receptor status of breast cancer in India: A study of 798 tumours. Breast 2000;9:267-70.
15. Pathak TB, Bashyal R, Pun CB, Shrestha S, Bastola S, Neupane S, *et al*. Estrogen and progesterone receptor expression in breast carcinoma. J Pathol Nepal 2011;1:100-3.
16. Lu X, Chen S, Huang S. A study on methodology and the criteria for positive immunohistostaining of estrogen and progesterone receptors in paraffin embedded sections of breast cancer. Zhonghua Bing Li Xue Za Zhi 1996;25:329-31.
17. Klimant E, Glurich I, Mukesh B, Onitilo AA. Blood type, hormone receptor status, HER2/neu status, and survival in breast cancer: A retrospective study exploring relationships in a phenotypically well-defined cohort. Clin Med Res 2011;9:111-8.
18. Ichikawa D, Handa K, Withers DA, Hakomori S. Histo-blood group A/B versus H status of human carcinoma cells as correlated with haptotactic cell motility: Approach with A and B gene transfection. Cancer Res 1997;57:3092-6.
19. Stamatakos M, Kontzoglou K, Safioleas P, Safioleas C, Manti C, Safioleas M. Breast cancer incidence in Greek women in relation to ABO blood groups and Rh factor. Int Semin Surg Oncol 2009;6:14.

Cite this article as: Haswani L, Suresh TN, Hemalatha A, Kumar ML, Bhaskaran A. Role of blood grouping as a prognostic marker in breast carcinoma its relationship with histological and hormonal prognostic markers. Clin Cancer Investig J 2014;3:497-501.

Source of Support: Nil, **Conflict of Interest:** None declared.